II. Support for the Amendments

Support for the amendments to the claims can be found throughout the specification, the drawings, and the claims as originally drafted. Support for the amendments to claims 11 and 27 can be found on, e.g., page 7, lines 24-26 of the specification. Support for the amendments to claims 12 and 28 can be found on, e.g., page 9, lines 22-27 of the specification. Support for new claims 40-42 can be found on, e.g., page 8, lines 14-15 of the specification. No new matter is added by this amendment.

III. Objection to Specification

The Examiner objected to hyperlinks in the specification per MPEP § 608.01. MPEP § 608.01 states that hyperlinks between the symbols "<>" or beginning with "http://" should not be allowed because electronic publication of the application will result in a hyperlink to another website.

Accordingly, the specification has been amended to delete any reference to "http://" or "www." Instead, the websites are referred to as "the website at ___," where the blank includes the website address after "www." In light of the amendment, Applicants respectfully request withdrawal of the objection.

IV. Objection to the claims

The Examiner objected to claims 11-12 and 27-28 because the claims contain references to trademarks. As amended, the claims no longer contain trademarks. Accordingly, withdrawal of the objections is respectfully requested.

V. Rejection under 35 U.S.C. § 102

Claims 1-4, 8-12, 18-21, 24-28, and 34-39 were rejected under 35 U.S.C. § 102(b) as allegedly anticipated by the RepeatMasker™ software. Specifically, the Examiner stated that the RepeatMasker™ software screens for low complexity repeats and the documentation for the software states that the resulting data can be used in BLAST™ searches or to design primers. With respect to claims 3, 8-10, 24-26 and 36-

38, the Examiner alleged that the **-div** option allows the user to limit masking with different percent divergences. Applicants respectfully traverse the rejection.

A. The RepeatMasker software does not teach or suggest using BLAST to identify primers that will amplify a limited number of sequences in a genome

The RepeatMasker software and documentation does not teach or suggest all of the elements of independent claims 1, 18 or 34. The RepeatMasker documentation does not teach or suggest performing a BLAST search and then designing primers for sequences that have a defined number of substantially similar sequences. The documentation does not suggest using the RepeatMasker software in combination with BLAST to identify non-repeated sequences for primer design. Indeed, there is no indication in the documentation that it is desirable to identify sequences with few or no substantially similar sequences thereby identifying sequences that have few or no cross reactivity in other parts of the genome.

Claim 1 provides four steps.

The first step involves identifying repeated sequences in a sequence.

The second step involves comparing the remainder of the sequence, i.e., the "repeat sequence-free subsequences" to a database to identify substantially similar sequences.

The third step involves using subsequences that only have a *defined number* of substantially similar sequences in the database as a template to design primers. The goal of this step is to *remove* sequences that have substantially similar sequences in the database, thereby only allowing for design of primers that will amplify truly unique sequences within the boundaries prescribed. Thus, primers would not be designed for sequences that have more than a defined number of substantially similar sequences.

The fourth step involves outputting the primer sequences.

Independent claims 18 and 34 comprise similar steps.

In contrast to the RepeatMasker software documentation, the present claims involve designing primers to amplify sequences that have few or no substantially similar sequences in a database. Moreover, the RepeatMasker software does not discuss any relation between BLAST or other database analysis and design of primers. Indeed, the documentation is drafted to suggest that the RepeatMasker software can be used in accordance with BLAST *or* used to design primers. There is no discussion of removing repeats, using BLAST *and* designing primers as a part of one process or method.

The RepeatMasker documentation does not even recognize the problem that is addressed by the present invention. The discussion of BLAST in the RepeatMasker documentation is directed to removing repeats outside of coding sequences to obtain more accurate BLAST data, thereby improving identification of related sequences in a database. The section of the RepeatMasker documentation directed to the use of BLAST searches cautions that "[t]he most common concern is of course if RepeatMasker ever masks coding regions." *See*, last paragraph of page 5 of RepeatMasker documentation. In contrast, in the context of the present invention, masking of a coding region that is repeated is a good thing, not a concern.

Therefore, the RepeatMasker software does not anticipate or render obvious any of the pending claims.

B. Claim 3 is not anticipated because the art does not teach or suggest design of primers for sequences that do not have substantially similar sequences in a database

Applicants submit that the Examiner has misread claim 3. The Examiner rejected claim 3 in light of the **-div** option, which allows users to choose the maximum amount of divergence used to identify repeats. Claim 3, in contrast, is directed to the embodiment where primers are only designed from sequences that do not have any substantially similar sequences in the database. Claim 3 does not address the percent similarity between a "repeat sequence-free subsequence" and a sequence in a database. Instead, claims 3 provides that only those sequences without *any* substantially similar

sequences in a database are selected for primer design. Therefore, claim 3 is not anticipated by, or even related to, the **-div** option in the RepeatMasker software.

C. The "-div" option in the RepeatMasker software does not address the same subject matter as recited in claims 8-10, 24-26 and 36-38

The Examiner rejected claims 8-10, 24-26 and 36-38 as anticipated by the "**-div**" option in the RepeatMasker software. Claims 8-10, 24-26 and 36-38 recite specific percent identity "cut-offs" for determining which sequences are "substantially identical" when comparing *repeat-free sequences* to a database. In other words, the subject matter of claims 8-10, 24-26 and 36-38 address a step that occurs *after* repeats have been removed. This is not what the "**-div**" option does.

The "**-div**" option allows the user to control the amount of masking (removal of common repeats) of a sequence. This process occurs *during* or *before* the removal of repeats. In fact it is part of the repeat-removal process. For example, by inputting a certain divergence (e.g., 18%), the software will only mask sequences that have repeats with that much divergence. In contrast, the subject matter of claims 8-10, 24-26 and 36-38 limits how *repeat-free* sequences are compared to a database. Thus, the **-div** option is unrelated to the subject matter of claims 8-10, 24-26 and 36-38.

For the reasons presented above, the RepeatMasker software cannot anticipate or render the present claims obvious.

VI. Rejection under 35 U.S.C. § 103

Claims 5-7, 13-17, 22, 23, and 25-33 were rejected under 35 U.S.C. § 103 as obvious over the RepeatMasker software in light of the Redasoft software. The Redasoft software was cited as describing web-based integrated primer ordering. Applicants respectfully traverse the rejection.

As described above, the RepeatMasker software does not teach or suggest any of the pending independent claims. While the Redasoft software may describe web-

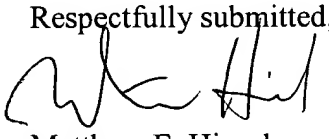
based integrated primer ordering, it does not compensate for the inadequacies of the RepeatMasker software. Accordingly, withdrawal of the rejection is respectfully requested.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,



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APPENDIX A

VERSION WITH MARKINGS TO SHOW CHANGES MADE

Please amend the last full paragraph on page 7 of the application as follows:

In a preferred embodiment, as depicted in Fig. 2, the process is carried out using any version of the RepeatMasker program (Arian Smit, University of Washington, Seattle, WA), such as RepeatMasker2. This program screens sequences for interspersed repeats that are known to exist in mammalian genomes, as well as for low complexity DNA sequences. The output of the program includes a detailed annotation of the repeats present in the query sequence, as well as a modified ("masked") version of the query sequence in which all the annotated repeats have been masked (*e.g.*, replaced by Ns). The RepeatMasker program is publicly available (*see, e.g.*, [http://repeatmasker.genome.washington.edu/)] the website at repeatmasker.genome.washington.edu/).

Please amend the paragraph bridging pages 7-8 of the application as follows:

Other usable programs include Censor (Jurka, *et al.* (1996) *Computers and Chemistry* 20:119-122; *see, e.g.*, the website at girinst.org/Censor_Server.html [http://www.girinst.org/Censor_Server.html]; Genetic Information Research Institute, California); Satellites or Repeats (Institut Pasteur, Paris; *see, e.g.*, the website at bioweb.pasteur.fr/seqanal/interfaces [http://bioweb.pasteur.fr/seqanal/interfaces]); and others.

Please amend the last full paragraph on page 9 of the application as follows:

Typically, the masked sequence (*i.e.*, collection of selected subsequences) will be compared with the genome database using a suitable algorithm such as BLAST (*see, e.g.*, the BLAST server at the National Center for Biotechnology Information[; http://www.ncbi.nlm.nih.gov/]). A BLAST or equivalent search will identify sequences within the genome that are homologous to the masked sequence, preferably ranked in order of similarity to each subsequence.

Please amend the paragraph bridging pages 10-11 of the application as follows:

A preferred example of algorithm that is suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul *et al., Nuc. Acids Res.* 25:3389-3402 (1977) and Altschul *et al., J. Mol. Biol.* 215:403-410 (1990), respectively. BLAST and BLAST 2.0 are used, with the parameters described herein, to determine percent sequence identity for the nucleic acids and proteins of the invention. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information [(http://www.ncbi.nlm.nih.gov/)]. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul *et al.*, *supra*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the

accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, M=5, N=-4 and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength of 3, and expectation (E) of 10, and the BLOSUM62 scoring matrix (*see* Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915 (1989)) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands.

Please amend the paragraph bridging pages 12-13 of the application as follows:

Typically, the primers will be designed not only based on the size of the product, but also taking into account any of a large number of considerations for optimal primer design, *e.g.*, to exclude potential secondary structures within the primers, with a desired T_m (that is preferably similar for each member of a pair of primers), to include additional sequences such as restriction sites to facilitate cloning of the amplified product, etc. Examples of suitable programs for designing (and analyzing potential primer sequences) include, but are not limited to, Primer3 (from the Whitehead Institute; website at genome.wi.mit.edu/cgi-bin/primer/primer3.cgi [http://www.genome.wi.mit.edu/cgi-bin/primer/primer3.cgi], PrimerDesign website at chemie.uni-marburg.de/~becker/pdhome.html [http://www.chemie.uni-marburg.de/~becker/pdhome.html)] Primer Express® Oligo Design Software (PE Biosystems), DOPE2 (Design of Oligonucleotide Primers website at dope.interactiva.de/ [http://dope.interactiva.de/]); DoPrimer (website at doprimer.interactiva.de [http://doprimer.interactiva.de]); NetPrimer (website at premierbiosoft.com/netprimer.html [http://www.premierbiosoft.com/netprimer.html]); Oligos-U-Like--Primers3 (website at path.cam.ac.uk/cgi-bin/primer3.cgi [http://www.path.cam.ac.uk/cgi-bin/primer3.cgi]); Oligo (v5.0); CpG Ware™ Primer

Design Software, PrimerCheck (website at chemie.uni-marburg.de/~becker/freeware/freeware.html#primercheck [http://www.chemie.uni-marburg.de/~becker/freeware/freeware.html#primercheck]), and others. General parameters for designing primers can be found in any of a large number of resources and publications, including Dieffenbach, *et al.*, in PCR Primer, A Laboratory Manual, Dieffenbach *et al.*, Ed., Cold Spring Harbor Laboratory Press, New York (1995), pp.133-155; Innis, *et al.*, in PCR protocols, A Guide to Methods and Applications, Innis, *et al.*, Ed., CRC Press, London (1994), pp. 5-11; Sharrocks, in PCR Technology, Current Innovations, Griffin, H.G., and Griffin, A.M, Ed., CRC Press, London (1994) 5-11.

IN THE CLAIMS:

11. (Once Amended) The method of claim 1, wherein said first process is executed using [Repeat Masker software] a software program that screens sequences for:

i. interspersed repeats that are known to exist in mammalian genomes and;

ii. low complexity DNA sequences.

12. (Once Amended) The method of claim 1, wherein said second process is executed using a sequence comparison algorithm [BLAST algorithm].

27. (Once Amended) The method of claim 18, wherein the identification of repeat sequences within said genomic region is performed using [Repeat Masker software] a software program that screens sequences for:

i. interspersed repeats that are known to exist in mammalian genomes and;

ii. low complexity DNA sequences.

28. (Once Amended) The method of claim 18, wherein the comparison of said at least one repeat sequence-free subsequence with said genome database is performed using a sequence comparison algorithm [BLAST algorithm].

40. (New) The method of claim 1, wherein the repeat-free subsequences are each at least 100 bp long.

41. (New) The method of claim 18, wherein the repeat-free subsequences are each at least 100 bp long.

42. (New) The computer program of claim 34, wherein each nucleotide sequence that does not contain any of the repeat sequences is at least 100 bp long.

APPENDIX B

CLAIMS PENDING WITH ENTRY OF AMENDMENT

1. A method for identifying oligonucleotide sequences suitable for the amplification of a unique sequence within a genomic region of interest, said method comprising the steps of:

executing a first process on a digital computer to identify repeat sequences that occur within said genomic region of interest;

executing a second process on a digital computer to compare repeat sequence free subsequences within said genomic region of interest to a nucleotide sequence database, whereby nucleotide sequences within said nucleotide sequence database that are substantially similar to said repeat sequence-free subsequences are identified;

executing a third process on a digital computer to identify oligonucleotide sequences that are suitable for use as primers in an amplification reaction to amplify a product within any of said repeat sequence-free subsequences for which a defined number of substantially similar sequences are identified in said nucleotide sequence database; and

outputting said oligonucleotide sequences.

2. The method of claim 1, wherein said genomic region is from a human genome.

3. The method of claim 1, wherein said number of substantially similar sequences is zero.

4. The method of claim 1, wherein said oligonucleotide sequences are outputted by displaying the sequences on a computer screen or on a computer printout.

5. The method of claim 1, wherein said oligonucleotide sequences are outputted by executing a fourth process on a digital computer to direct the synthesis of oligonucleotide primers comprising said oligonucleotide sequences.

6. The method of claim 5, wherein said computer directs the synthesis of said oligonucleotide primers by ordering said synthesis from an external source.

7. The method of claim 5, wherein said computer is in communication with an oligonucleotide synthesizer, and wherein said computer directs the synthesis of said oligonucleotide primers by said synthesizer.

8. The method of claim 1, wherein said substantially similar sequences are at least about 50% identical to said repeat sequence-free subsequences.

9. The method of claim 1, wherein said substantially similar sequences are at least about 70% identical to said repeat sequence-free subsequences.

10. The method of claim 1, wherein said substantially similar sequences are at least about 90% identical to said repeat sequence-free subsequences.

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11. (Once Amended) The method of claim 1, wherein said first process is executed using a software program that screens sequences for:

i. interspersed repeats that are known to exist in mammalian genomes and;

ii. low complexity DNA sequences.

12. (Once Amended) The method of claim 1, wherein said second process is executed using a sequence comparison algorithm.

13. The method of claim 1, wherein said third process is executed using Primer3 software.

14. The method of claim 5, further comprising producing an amplification product using said oligonucleotide primers.

15. The method of claim 14, wherein said amplification product is a FISH probe.

16. The method of claim 15, wherein said FISH probe is fluorescently labeled.

17. The method of claim 14, wherein said amplification product is an array CGH target.

18. A method for identifying oligonucleotide sequences suitable for the amplification of a unique sequence within a genomic region of interest, said method comprising the steps of:

analyzing a genomic nucleotide sequence that encompasses said genomic region of interest to identify repeat sequences within said genomic region;

comparing at least one repeat sequence-free subsequence within said genomic nucleotide sequence to a nucleotide sequence database to identify sequences within said database that are substantially similar to said repeat sequence-free subsequence;

for at least one of said repeat sequence-free subsequences for which a defined number of substantially similar sequences are identified within said nucleotide sequence database, selecting oligonucleotide sequences that are suitable for use as primers in an amplification reaction to amplify a product within said repeat sequence-free subsequence.

19. The method of claim 18, wherein said genomic region is from a human genome.

20. The method of claim 18, wherein said defined number of substantially similar sequences is zero.

21. The method of claim 18, further comprising displaying said oligonucleotide sequences on a computer screen or on a computer printout.

22. The method of claim 18, further comprising directing the synthesis of oligonucleotide primers comprising said oligonucleotide sequences.

23. The method of claim 22, wherein said synthesis is directed by ordering the synthesis of said primers from an external source.

24. The method of claim 18, wherein said substantially similar sequences are at least about 50% identical to said repeat sequence-free subsequences.

25. The method of claim 18, wherein said substantially similar sequences are at least about 70% identical to said repeat sequence-free subsequences.

26. The method of claim 18, wherein said substantially similar sequences are at least about 90% identical to said repeat sequence-free subsequences.

27. (Once Amended) The method of claim 18, wherein the identification of repeat sequences within said genomic region is performed using a software program that screens sequences for:

i. interspersed repeats that are known to exist in mammalian genomes and;

ii. low complexity DNA sequences.

28. (Once Amended) The method of claim 18, wherein the comparison of said at least one repeat sequence-free subsequence with said genome database is performed using a sequence comparison algorithm.

29. The method of claim 18, wherein said oligonucleotide sequences are selected using Primer3 software.

30. The method of claim 22, further comprising generating an amplification product using said oligonucleotide primers.

31. The method of claim 30, wherein said amplification product is a FISH probe.

32. The method of claim 31, wherein said FISH probe is fluorescently labeled.

33. The method of claim 30, wherein said amplification product is an array CGH target.

34. A computer program product designing and outputting oligonucleotide sequences suitable for use as primers to amplify unique sequences within a genomic region of interest, said computer program product comprising:

a storage structure having computer program code embodied therein, said computer program code comprising:

computer program code for causing a computer to analyze a nucleotide sequence encompassing said genomic region of interest to identify repeat sequences within said nucleotide sequence;

computer program code for causing a computer to, for each subsequence of said nucleotide sequence that does not contain any of said repeat sequences, compare said subsequence against a nucleotide sequence database to identify nucleotide sequences within said database that are substantially similar to said subsequence;

computer program code for causing a computer to, for each of said subsequences for which a defined number of substantially similar sequences are found in said database, identify oligonucleotide sequences suitable for use as primers in an amplification reaction to amplify a product within said subsequence; and

computer program code for outputting said oligonucleotide sequences.

35. The method of claim 34, wherein said defined number of substantially similar sequences is zero.

36. The method of claim 34, wherein said substantially similar sequences are at least about 50% identical to said subsequences.

37. The method of claim 34, wherein said substantially similar sequences are at least about 70% identical to said subsequences.

38. The method of claim 34, wherein said substantially similar sequences are at least about 90% identical to said subsequences.

40. (New) The method of claim 1, wherein the repeat-free subsequences are each at least 100 bp long.

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41. (New) The method of claim 18, wherein the repeat-free subsequences are each at least 100 bp long.

42. (New) The computer program of claim 34, wherein each nucleotide sequence that does not contain any of the repeat sequences is at least 100 bp long.